DEHYDRATION AND CLEARING

DEHYDRATION

AIM: to **remove fixative and water** from the tissue and replacing them with dehydrating fluid in **preparation for impregnation.**

- Dehydrating fluids are generally used in increasing strengths.
- **INCREASING STRENGTHS** = all the aqueous tissue fluids are removed but with little disruption to the tissue due to diffusion currents.
- For delicate tissues, particularly embryonic and animal tissues, it is recommended to start processing with **30% ethyl alcohol** (embryonic & animal tissues)

CHARACTERISTICS OF AN IDEAL DEHYDRATING SOLUTION:

1. It should dehydrate rapidly without producing considerable shrinkage or distortion of tissues

2. It should not evaporate very fast

3. It should be able to dehydrate even fatty tissues

4. It should not harden tissues excessively

5. It should not remove stains

6. It should not be toxic to the body

7. It should not be a fire hazard

COMMONLY USED DEHYDRATING AGENT

1. Alcohol – **MOST COMMON**

a. **Ethanol** = for routine dehydration of tissues.

= BEST DEHYDRATING AGENT

b. **Methyl alcohol** = employed for blood and tissue films

c. **Butyl alcohol** = utilized in plant and animal micro techniques.

d. **Industrial methylated spirit (denatured alcohol)** = ethanol + small amount of methanol; used in the same way as ethanol

e. **Isopropyl alcohol** = many of the processing methods for use in a microwave oven recommend this agent.

2. Acetone – **BOTH fixative and dehydrating agent.**

3. Dioxane (Diethylene dioxide) – **BOTH dehydrating and clearing agent**

4. Cellosolve (Ethylene glycol monoethyl ether) – **BOTH dehydrating and clearing agent**

5. THF (Tetrahydrofuran) – **BOTH dehydrating agent and clearing agent**

6. Triethyl phosphate

ADDITIVES TO DEHYDRATING AGENT:

1. 4% phenol + each 95% ethanol baths

acts as a tissue softener for hard tissues such as tendons, nails, or dense fibrous tissues.

2. Anhydrous copper sulfate = can act as BOTH dehydrating agent and an indicator of water content of the last bath (100% ETOH).

NOTES TO REMEMBER WHEN USING ALCOHOL •Strength of alcohol depend upon the size, and the nature of the tissue and fixative used

•Length of storage

•Temperature

•Anhydrous copper sulfate, serves as an indicator that dehydration is complete (white blue)

•Xylene: turns milky

CLEARING

CLEARING AGENT

1. It should be **miscible with alcohol** to remove dehydrating agent from the tissues

2. Generally clearing agent should be **miscible with paraffin wax** to facilitate embedding,

3. Should be **miscible with Canada balsam and other resins** for during mounting,

4. Should **make tissue transparent after staining** due to their high refractive index

5. Most clearing agents are **flammable liquids** that requires proper handling

6. **Viscosity affects speed of penetration** of the clearing agent

7. **Prolonged exposure** to most clearing agents causes the tissue to become **brittle and more difficult to cut**

XYLENE/XYLOL

- **Colorless** clearing agent
- **MOST COMMONLY USED** in routine procedures
- Clearing time: **30 – 60 MINUTES**
- Xylene turns **MILKY** when tissues are **incompletely dehydrated**

TOLUENE

- **Substitute** for xylene or benzene
- Time for clearing **1-2 hours**
- Also used **both** for **embedding and mounting** process

BENZENE

- Recommended for **URGENT BIOPSIES**
- **CARCINOGENIC**
- Can cause **damage to BM & Aplastic anemia**

CHLOROFORM

- **Slower** in action
- May cause **HEPATOTOXICITY**
- **Does not make tissue translucent** but removes alcohol

CEDARWOOD OIL

- Recommended for **CNS TISSUE AND CYTOLOGY**
- **No tissue distortion** even if left in oil
- **Very expensive**
- Clearing agent for **paraffin and celloidin sections**

ANILINE OIL

- Not for routine purposes
- Recommended for **embryos, insects**, and **VERY DELICATE TISSUES**

CLOVE OIL

- Causes **MINIMUM SHRINKAGE**
- Becomes **adulterated**

CARBON TETRACHLORIDE

- Similar to chloroform but **cheaper**
- **Highly toxic** upon **prolonged exposure**

METHYL BENZOATE AND METHYL SALICYLATE

- **Slow** acting
- For **double embedding techniques**

GLYCERINE/GUM SYRUP

- **No dealcoholization** but **make the tissues clearer**

OTHER CLEARING AGENTS

1. Oil of bergamot
2. Oil of origanum
3. Oil of wintergreen
4. Carbon disulfide
5. Carbol xylene
6. Terpineol
7. Phenol
8. High test aviation lead free gasoline